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MAPPING ALLOREACTIVE T CELL EPITOPES ON THE RHESUS D PROTEIN. L.-M. Stott*, D.W.L. Wilson*, R.N. Barker*, S.J. Urbaniak* (Intr. by I.M. Franklin). *Dept. Medicine & Therapeutics, University of Aberdeen, AB25 2ZD, UK.*

The Rhesus (Rh) D antigen is important in transfusion medicine and is the major target in haemolytic disease of the newborn (HDN). The current program to prevent alloimmunisation of women bearing RhD-incompatible children is threatened by a shortage of anti-RhD immunoglobulin donors. The development an improved or alternative strategy is dependent on characterisation of the immune response to RhD. Since virtually nothing is known about the helper response, the aim of the current work was to identify T cell epitopes on the RhD protein. Peripheral blood mononuclear cells (PBMC) were obtained from 24 individuals who had developed anti-RhD alloantibodies following natural or deliberate immunisation, and from eight alloantibody-negative, RhD-negative control donors. A panel of 68 overlapping synthetic 15-mer peptides, spanning the sequence of the RhD protein, was screened for the ability to stimulate recall prolifcration of T cells in cultures of the PBMC. The results show that PBMC from 17 of the 24 alloimmune donors, but from only two of the eight controls, responded to the RhD peptides. The pattern of stimulatory peptides varied between alloimmune donors and showed some evolution over time. However, particular peptides were commonly stimulatory, including numbers 2, 12, 12a, 15a, 18a and 28, which each elicited a response in over 40% of donors. These sequences, with the exception of 2 and 28, contain RhD-specific polymorphisms. It is currently being determined how the pattern of stimulatory peptides varies with HLA allotype, and preliminary results have revealed significantly related profiles in donors who share DRB1*15.01. Stimulatory peptides were found throughout the intracellular, transcellular and extracellular domains of the RhD protein, a distribution that was not unexpected, since helper T cells recognise fragments of antigen processed by antigen presenting cells. In conclusion, we have identified peptides containing putative helper T cell epitopes that may be important in anti-RhD alloantibody production. The characterisation of these epitopes is a key step in development of safe, synthetic immunogens for anti-RhD immunoglobulin donors, and opens the way for the evaluation of peptide immunotherapy as a novel approach to the prevention of HDN.